

Molecular Mechanisms in the Hematopoietic Stem Cell Niche

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A stem cell together with its surrounding microenvironmental cells represents the stem cell niche. A niche may be defined functionally as a dynamic multi-cellular and structural unit that balances self-renewal and cell fate decisions. As such, the stem cell niche must enable the spatiotemporal interactions between stem cells and the cells representing their microenvironment in such a way as to provide for the lifelong production of mature cell populations. We have taken a functional genomics approach to elucidate the molecular signals emanating from the microenvironmental elements in the niche and to measure crosstalk among niche components. As a first step, we generated a cDNA library enriched for molecules preferentially expressed by the mouse fetal liver derived stromal cell line, AFT024. We suggest that AFT024 cells represent a microenvironmental component of the niche unit. We have shown that these cells maintain murine HSCs with long-term competitive repopulating ability and, also, human HSC activity in long-term *in vitro* assays and *in vivo* in xenogeneic transplant models. The annotated sequence set is available to the public in the Stromal Cell Database (StroCDB; <http://stromalcell.princeton.edu>). From this set we have selected both known and novel gene products that are predicted to be secreted or cell surface molecules for gain (over expression) and loss (interfering RNA) of function studies. The Wnt signaling pathway is highly represented in StroCDB and in comparative microarray studies with AFT024 cells and HSC. This pathway has been implicated in the self-renewal and proliferation of multiple stem cells including HSC. To study the role of Wnt signaling in the AFT024 system, we expressed a Wnt inhibitor, WIF-1, in these cells and observed a decrease in stem cell support. At present, we are developing a Wnt reporter to further dissect the pathway and to observe active Wnt signaling in AFT024/HSC co-cultures. Studies with this reporter will be extended to the WIF-1 expressing AFT024 cells. We are also generating a transgenic mouse with WIF-1 under the control of the osteoblast specific col2.3 promoter to study inhibition of Wnt signaling in the native niche. An additional approach investigates the crosstalk among niche components. Stem cells have been recovered after co-culture with AFT024 and analyzed using microarrays. AFT024 cells from these cultures have also been analyzed in a similar manner. We are examining the data for correlated alterations in the expression of signaling pathway components and other potentially relevant molecules. Collectively, these types of functional genomics studies will help elucidate the molecular mechanisms in extrinsic stem cell regulatory networks.